

AMENDMENTS TO THE SPECIFICATION:

Please replace the paragraph beginning on page 5, line 1, and ending on page 5, line 16, with the following paragraph:

In addition, when a peptide fingerprint of an unknown protein is obtained, it can be compared to a database of known proteins to assist in the identification of the unknown protein using mass spectrometry (W.J. Henzel et al., *Proc. Natl. Acad. Sci. USA* 90:5011-5015, 1993; D. Fenyo et al., *Electrophoresis* 19:998-1005, 1998). A variety of computer software programs to facilitate these comparisons are accessible via the Internet, such as Protein Prospector (Internet site: prospector.uscf.edu), Multident (Internet site: www.expasy.ch/sprot/multiident.html), ~~www.expasy.ch/sprot/multiident.html~~, PeptideSearch (Internet site: www.mann.embl-heidelberg.de...deSearch/FR_PeptideSearch_Form.html), ~~www.mann.embl-heidelberg.de...deSearch/FR_PeptideSearch_Form.html~~, and ProFound (Internet site: www.chait.sgi.rockefeller.edu/cgi-bin/protidfrag.html), ~~www.chait.sgi.rockefeller.edu/cgi-bin/protidfrag.html~~). These programs allow the user to specify the cleavage agent and the molecular weights of the fragmented peptides within a designated tolerance. The programs compare these molecular weights to protein molecular weight information stored in databases to assist in determining the identity of the unknown protein. Accurate information concerning the number of fragmented peptides and the precise molecular weight of those peptides is required for accurate identification. Therefore, increasing the accuracy in determining the number of fragmented peptides and their molecular weight should result in enhanced likelihood of success in the identification of unknown proteins.

Please replace the paragraph beginning on page 5, line 17, and ending on page 5, line 25, with the following paragraph:

In addition, peptide digests of unknown proteins can be sequenced using tandem mass spectrometry (MS/MS) and the resulting sequence searched against databases (J.K. Eng, et al., *J. Am. Soc. Mass Spec.* 5:976-989 (1994); M. Mann and M. Wilm, *Anal. Chem.* 66:4390-4399 (1994); J.A. Taylor and R.S. Johnson, *Rapid Comm. Mass Spec.* 11:1067-1075 (1997)). Searching programs that can be used in this process exist on the Internet, such as Lutefisk 97 (Internet site www.lsbcc.com:70/Lutefisk97.html), ~~www.lsbcc.com:70/Lutefisk97.html~~, and the Protein Prospector, Peptide Search and ProFound programs described above. Therefore, adding the sequence of a gene and its predicted protein sequence and peptide fragments to a sequence database can aid in the identification of unknown proteins using tandem mass spectrometry.

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Please replace the paragraph beginning on page 17, line 20, and ending on page 18, line 1, with the following paragraph:

Other derivatives include covalent or aggregative conjugates of the polypeptides with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. Examples of fusion proteins are discussed below in connection with oligomers. Further, fusion proteins can comprise peptides added to facilitate purification and identification. Such peptides include, for example, poly-His or the antigenic identification peptides described in U.S. Patent No. 5,011,912 and in Hopp et al., *Bio/Technology* 6:1204, 1988. One such peptide is the FLAG[®] peptide, Asp-Tyr-Lys-Asp-Asp-Asp-Lys (SEQ ID NO:5), which is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. A murine hybridoma designated 4E11 produces a monoclonal antibody that binds the FLAG[®] peptide in the presence of certain divalent metal cations, as described in U.S. Patent 5,011,912, hereby incorporated by reference. The 4E11 hybridoma cell line has been deposited with the American Type Culture Collection under accession no. HB 9259. Monoclonal antibodies that bind the FLAG[®] peptide are available from Eastman Kodak Co., Scientific Imaging Systems Division, New Haven, Connecticut.

Please replace the paragraph beginning on page 23, line 10, and ending on page 23, line 15, with the following paragraph:

Certain leucine zipper moieties preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (*FEBS Letters* 344:191, 1994) and in U.S. Patent 5,716,805, hereby incorporated by reference in their entirety. This lung SPD-derived leucine zipper peptide comprises the amino acid sequence Pro Asp Val Ala Ser Leu Arg Gln Gln Val Glu Ala Leu Gln Gly Gln Val Gln His Leu Gln Ala Ala Phe Ser Gln Tyr (SEQ ID NO:6).

Please replace the paragraph beginning on page 23, line 16, and ending on page 23, line 20, with the following paragraph:

Another example of a leucine zipper that promotes trimerization is a peptide comprising the amino acid sequence Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser Lys Ile Tyr His Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu Arg (SEQ ID NO:7), as described in U.S. Patent 5,716,805. In one alternative embodiment, an N-terminal Asp residue is added; in another, the peptide lacks the N-terminal Arg residue.

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Please replace the paragraph beginning on page 37, line 10, and ending on page 37, line 21, with the following paragraph:

For example, chromosomes can be mapped by radiation hybridization. PCR is performed using the Whitehead Institute/MIT Center for Genome Research Genebridge4 panel of 93 radiation hybrids (http://www-genome.wi.mit.edu/ftp/distribution/human_STS_releases/july97/rhmap/genebridge4.html) (~~http://www-genome.wi.mit.edu/ftp/distribution/human_STS_releases/july97/rhmap/genebridge4.html~~). Primers are used which lie within a putative exon of the gene of interest and which amplify a product from human genomic DNA, but do not amplify hamster genomic DNA. The results of the PCRs are converted into a data vector that is submitted to the Whitehead/MIT Radiation Mapping site on the internet (<http://www-seq.wi.mit.edu>) (~~<http://www-seq.wi.mit.edu>~~). The data is scored and the chromosomal assignment and placement relative to known Sequence Tag Site (STS) markers on the radiation hybrid map is provided. The following web site provides additional information about radiation hybrid mapping: http://www-genome.wi.mit.edu/ftp/distribution/human_STS_releases/july97/07-97.INTRO.html (~~http://www-genome.wi.mit.edu/ftp/distribution/human_STS_releases/july97/07-97.INTRO.html~~).

Please replace the paragraph beginning on page 53, line 15, and ending on page 53, line 26, with the following paragraph:

As set forth above, a polypeptide or peptide fingerprint can be entered into or compared to a database of known proteins to assist in the identification of the unknown protein using mass spectrometry (W.J. Henzel et al., Proc. Natl. Acad. Sci. USA 90:5011-5015, 1993; D. Fenyo et al., Electrophoresis 19:998-1005, 1998). A variety of computer software programs to facilitate these comparisons are accessible via the Internet, such as Protein Prospector (Internet site: prospector.uscf.edu), Multident (Internet site: www.expasy.ch/sprot/multiident.html) (~~www.expasy.ch/sprot/multiident.html~~), PeptideSearch (Internet site: www.mann.embl-heidelberg.de/deSearch/FR_PeptideSearch_Form.html) (~~www.mann.embl-heidelberg.de/deSearch/FR_PeptideSearch_Form.html~~), and ProFound (Internet site: www.chait.sgi.rockefeller.edu/cgi-bin/prot_id_frag.html) (~~www.chait.sgi.rockefeller.edu/cgi-bin/prot_id_frag.html~~). These programs allow the user to specify the cleavage agent and the molecular weights of the fragmented peptides within a designated tolerance. The programs compare observed molecular weights to predicted peptide molecular weights derived from sequence databases to assist in determining the identity of the unknown protein.

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Please replace the paragraph beginning on page 53, line 27, and ending on page 54, line 2, with the following paragraph:

In addition, a polypeptide or peptide digest can be sequenced using tandem mass spectrometry (MS/MS) and the resulting sequence searched against databases (J.K. Eng, et al., J. Am. Soc. Mass Spec. 5:976-989 (1994); M. Mann and M. Wilm, Anal. Chem. 66:4390-4399 (1994); J.A. Taylor and R.S. Johnson, Rapid Comm. Mass Spec. 11:1067-1075 (1997)). Searching programs that can be used in this process exist on the Internet, such as Lutefisk 97 (Internet site www.lsbcc.com:70/Lutefisk97.html), and the Protein Prospector, Peptide Search and ProFound programs described above.

Please replace the paragraph beginning on page 59, line 24, and ending on page 60, line 19, with the following paragraph:

Analysis was performed by an initial round of PCR followed by a second round of PCR using nested primers.

FIRST ROUND: Primers: 5' sense = BRR464.26 (just upstream of coding region, in 5' UTR)

3' antisense = BRR453.26 (within coding region, near 3' end)

BRR464.26 5' GGGAGTCTACACCCTGTGGAGCTCAA 3' (SEQ ID NO:8)

BRR453.26 5' CTGCTGGAAGTAGAAGTCTGTGATGG 3' (SEQ ID NO:9)

Reaction conditions (per 50ul reaction):

5ul each 1st strand cDNA (or 5ul H2O for negative control or 100ng human genomic DNA), 12.5 pmol each primer
-200uM each dNTP, 0.5ul of a 16:1 mixture of KlenTaq1 and Vent polymerases, 50mM Tris-HCl, pH 9.2 -3.5mM MgCl₂, 16mM (NH₄)₂SO₄, and 150ug/ml BSA

Cycling parameters:

1 cycle 98°C, 3min; 58°C, 45sec; 72°C, 45sec
28 cycles 98°C, 45sec; 58°C, 45sec; 72°C, 45sec
1 cycle 98°C, 45sec; 58°C, 45sec; 72°C, 45sec

SECOND ROUND: Reactions from first round were diluted 1:50 in TE and 1µl of each dilution amplified with nested primers.

Primers: 5' sense = BRR463.30 (spans initiating ATG; downstream of and overlapping first sense primer). 3' antisense = BRR462.28 (within coding region, near 3' end; downstream of first antisense primer).

BRR463.30 5' GGAGCTCAAGATGGTCCTGAGTGGGGCGCT 3' (SEQ ID NO:10)

BRR462.28 5' GCATTCCAGCCACCATTCTCGGGAAGCT 3' (SEQ ID NO:11)

Reaction conditions and cycling parameters were identical to that of the first round.

10µl of each reaction from first and second round PCRs were separated by electrophoresis on 1.2% agarose gels. Products were visualized by UV light following staining with ethidium bromide. Expression was detected in lymph node, thymus, tonsil, brain, placenta, lung, skeletal muscle, prostate, and testis.

Please replace the paragraph beginning on page 60, line 23, and ending on page 60, line 32, with the following paragraph:

The poly His tag is used to bind the recombinant protein to Nickel-NTA resin (manufactured by Qiagen, <http://www.qiagen.com/> ~~http://www.qiagen.com/~~) according to the manufacturer's instructions. The resin is washed with 30 column volumes of 20 mM NaPO₄ pH 7.4 + 300 mM NaCl + 5 mM Imidazole. The recombinant protein is then eluted using increasing concentrations of Imidazole. Initially a gradient of 5-20mM Imidazole 20 mM NaPO₄ pH 7.4 + 300 mM NaCl is used, followed by 20mM Imidazole 20 mM NaPO₄ pH 7.4 + 300 mM NaCl, followed by a gradient of 20-100mM Imidazole 20 mM NaPO₄ pH 7.4 + 300 mM NaCl, followed by 100mM Imidazole 20 mM NaPO₄ pH 7.4 + 300 mM NaCl, followed by 500mM Imidazole 20 mM NaPO₄ pH 7.4 + 300 mM NaCl. Fractions are collected and analysed by SDS-PAGE to identify those containing the recombinant protein.

Please replace the sequence listing previously filed on July 10, 2000, with the attached substitute sequence listing entitled "SEQUENCE LISTING."